

Electrostatic theory of viral self-assembly: a toy model

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Viruses self-assemble from identical capsid proteins and their genome consisting, for example, of a long single stranded (ss) RNA. For a big class of $T = 3$ viruses capsid proteins have long positive N-terminal tails. We explore the role played by the Coulomb interaction between the brush of positive N-terminal tails rooted at the inner surface of the capsid and the negative ss RNA molecule. We show that viruses are most stable when the total contour length of ss RNA is close to the total length of the tails. For such a structure the absolute value of the total RNA charge is approximately twice larger than the charge of the capsid. This conclusion agrees with structural data.

Unlike living cells, viruses do not have any metabolic activity, which may mean that they are in the state of thermal equilibrium. This is one of the reasons why the statistical physics can be used for understanding of viruses. The structure of viruses is also dramatically simple. Inside the protein capsid each virus carries its genome, which consists of one or more DNA or RNA molecules and is used for reproduction in host cells. The focus of this letter is on viruses with single stranded RNA (ss RNA) genomes. Detailed image reconstruction of apparently spherical viruses reveals their icosahedral symmetry. This is why such a virus capsid can be viewed as a curved two-dimensional crystal closed on itself [1, 2, 3].

Here we concentrate on the viruses of the so called $T = 3$ class, in which a capsid is made of precisely 180 identical proteins, or of 60 triangular blocks consisting of three proteins each (see Fig. 1). In-vitro studies of solutions of capsid proteins and RNA molecules of a given virus show that under the biological pH and salinity they can spontaneously self-assemble into infectious viruses [4, 5, 6]. This letter focuses on the energetics of this amazing protein-RNA self-assembly. In addition to hydrophobic attraction between the proteins it is driven by strong Coulomb attraction between capsid proteins and RNA molecules [3, 4]. Indeed, ss RNA is strongly negatively charged. Its backbone has one negative phosphate per nucleotide or per 0.65 nm. We denote the total ss RNA charge of a virus particle as $-Q_r$. According to Tab. I of $T = 3$ viruses Q_r is about several thousand in units of the proton charge. On the other hand, for many viruses their capsid proteins carry substantial net positive charge q_p , which can reach 17. The net positive charge of the capsid of a $T = 3$ virus $Q_c = 180q_p$ can, therefore, reach 3000. Although, in biological conditions the protein-RNA interaction is screened by monovalent salt at the Debye-Huckel screening radius r_s , attraction energy of such big charges is still very large.

A dramatic feature of the group A of $T = 3$ viruses collected in the upper part of Tab. I is that almost all the capsid protein charge is concentrated in the N-terminal tail located inside the capsid (Fig. 1). We define such an N-terminal tail as the flexible sequence of amino acids, which starts from the N-terminus of the protein and ends

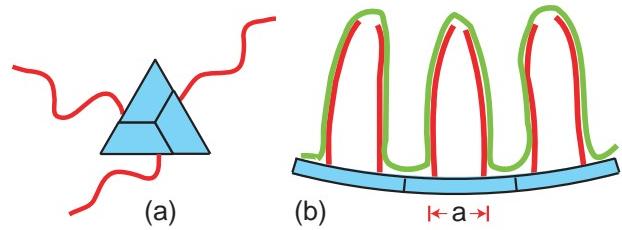


FIG. 1: (color online) Schematic sketch of the protein capsid assembly. (a) Triangular block made of three proteins (blue) with their positive flexible N-terminal tails (red). (b) The brush of positive N-terminal tails rooted at the inner surface of the capsid made of triangular blocks. The ss RNA (green) strongly interacts with the tails and keeps all the blocks together.

at the first α -helix or β -sheet. It looks like evolution created cationic N-terminal tails for the strong interaction with ss RNA genome (Fig. 1b).

In this letter we concentrate on the electrostatic interaction of the ss RNA with the brush of tails of a group A virus (see Fig. 1b). In particular we want to understand a remarkable fact that for these viruses the absolute value of the ss RNA charge Q_r is substantially larger than the total charge of the capsid $Q_c = 180q_p$. The charge inversion ratios $R = Q_r/Q_c$ for them are given in Tab. I. They are scattered with the median value 1.8. This raises a challenging question whether such ratio can be obtained by minimizing free energy of the virus [9] with respect to RNA length. The positive answer to this question was recently given in the framework of the simplest model where positive protein charges are uniformly smeared on the internal surface of the capsid, while the ss RNA is adsorbed on this surface as a negative polyelectrolyte [9]. As we see from Tab. I capsid charges of all the group A viruses are concentrated in the tails. That is why we suggest an alternative model of virus self-assembly, namely adsorption of ss RNA on a brush of flexible positive tails, rooted on a neutral surface. Minimizing the free energy of such self-assembly with respect to the total ss RNA length we arrive at the theoretical charge inversion ratios \mathfrak{R} , which are quite close to the factual ones R .

TABLE I: The absolute value of ss RNA charge Q_r , the charge of the capsid protein q_p , the N-terminal tail charge q_t , the number of amino acids in the tail N_t , the ratio of the linear charge densities (in fully stretched state) of the ss RNA η_r and the tail η_t , the ratio N_d/N_t , where N_d is number of amino acids in disordered part of the tail, the actual and predicted charge inversion ratios R and \Re . The data are obtained from Refs. [7, 8]. In the group A most of the capsid charges are concentrated in the tails. In the group B the protein charges are large but the tails are practically neutral. In the group C the charges of both capsid proteins and tails are very small.

Virus	Q_r	q_p	q_t	N_t	$\frac{\eta_r}{\eta_t}$	$\frac{N_d}{N_t}$	R	\Re
Group A								
Brome Mosaic	3030	10	9	48	2.8	0.44	1.7	2.8
Cowpea Chlorotic Mottle	2980	7	9	49	2.8	0.63	2.4	2.8
Cucumber Mosaic	3214	15	12	66	2.9	0.59	1.2	2.9
Tomato Aspermy	3391	14	12	67	2.9	0.55	1.3	2.9
Nodamura	4540	13	18	52	1.5	0.79	1.9	1.8
Pariacoto	4322	13	14	47	1.8	0.61	1.8	1.9
Sesbania Mosaic	4149	6	6	57	5.0	0.89	3.8	5.0
Rice Yellow Mottle	4450	17	12	52	2.3	0.79	1.5	2.3
Southern Bean Mosaic	4136	16	14	58	2.2	0.89	1.4	2.2
Cocksfoot Mottle	4082	15	13	54	2.2	0.88	1.5	2.2
Carnation Mottle	4003	10	11	81	3.9	1.0	2.2	3.9
Tobacco Necrosis	3700	9	10	79	4.1	0.90	2.3	4.1
Tomato Bushy Stunt	4776	12	13	92	3.7	0.91	2.2	3.7
Group B								
Dengue	10735	9	1	7				
Immature Yellow Fever	10862	14	1	7				
Immature Dengue-2 prM	10703	15	1	7				
Group C								
Norwalk	7654	4	-2	28				
Native Calicivirus	7500	1	-4	26				
Bacteriophage Q Beta	4215	3	1	3				
Bacteriophage Ga	3466	3	0	3				
Bacteriophage MS2	3569	1	0	4				
Bacteriophage Fr	3575	1	0	4				
Bacteriophage Pp7	3588	1	0	3				
Bacteriophage alpha3	6087	-4	-1	8				
Turnip Yellow Mosaic	6318	0	-2	29				
Desmodium yellow mottle	6300	5	1	29				
Physalis Mottle	6673	4	-1	28				

We call our model a toy model because we start from the following two simplifications. (i) First, similar to Ref. [9] we neglect hydrogen bonds between ss RNA bases which lead to the secondary structure of ss RNA. (ii) Second, we assume that each tail is free (does not stick to the capsid surface). Actually for some tails, their part close to the tail root sticks to the capsid surface [10]. Only this part of the N-terminal tail is seen in the X-ray images of the crystallized viruses, while the rest of the tail is missing. Missing part of the tail strongly fluctuates and is called disordered. We call N_d the average number of amino acids in the disordered (free) part of the tail. Ratios N_d/N_t are given in Tab. I. We see that on average 76% of the tail length is free. In our toy model we assume that $N_d = N_t$.

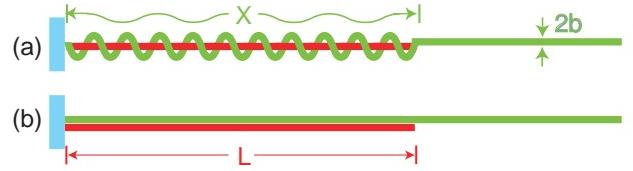


FIG. 2: (color online) Complexes of the long ss RNA (green) with a cationic tail (red) rooted on the internal surface of capsid (blue). L is the length of the tail, X is the length of RNA piece, which complexes with the tail. The structure and the magnitude of X depends on the ratio between the charge densities of the tail and the ss RNA. (a) $X > L$, when $\eta_r < 2\eta_t$; (b) $X = L$, when $\eta_r > 2\eta_t$.

Let us first consider interaction of a homo-polymeric ss RNA with a single free cationic N-terminal tail rooted at the neutral internal surface of the capsid (Fig. 2a). We assume that in fully stretched state each tail has length L and the positive linear charge density η_t , while the very long ss RNA in fully stretched state has the negative linear charge density $-\eta_r$. The ss RNA piece of the length $X \geq L$ complexes with the tail. Both polymers are modelled as worm-like chains with the same radius b , which is simultaneously of the order of their bare persistence length p_0 (which does not include Coulomb self-repulsion). The third important assumption of our toy model is that (iii) the solution has a moderate salt concentration, so that $b \ll r_s \ll L$. We argue below that even this assumption does not change our results qualitatively.

Due to the strong Coulomb repulsion inside the overcharged complex, the strongly negatively charged ss RNA has a relatively large persistence length $p \sim r_s^2/p_0$ (see Refs. [11, 12, 13]), so that its Coulomb energy can be estimated as the energy of a rigid cylinder of the radius b . Same is true for the complex of the N-terminal tail and the ss RNA, which as we will see has the large negative linear charge density η^* . Self-repulsion of these negative charges makes the complex locally stretched, so that its total length equals L . Therefore, $\eta^* = (-X\eta_r + L\eta_t)/L$. The tail-RNA complex with the long ss RNA shown in the Fig. 2a has the large electrostatic energy. Therefore, the contribution to the free energy F from configurational entropy plays a minor role and can be neglected. Since $r_s \ll L \leq X$, the Coulomb interaction is truncated at r_s . As a result, we obtain the following simple expression for the X -dependent part of the free energy

$$F(X) = L \left(\frac{-X\eta_r + L\eta_t}{L} \right)^2 \ln \left(\frac{r_s}{b} \right) - X\eta_r^2 \ln \left(\frac{r_s}{b} \right). \quad (1)$$

The first term represents the self-energy of the overcharged N-terminal tail (the complex), while the second term represents the loss of the electrostatic energy of the ss RNA segment with length X . Here we neglect the Coulomb repulsion between the complex and

the rest of the ss RNA because $r_s \ll L, X$. Minimizing $F(X)$ with respect to X , we find the optimal $X = X_0 = (\eta_t/\eta_r + 1/2)L$, and the linear charge density of the complex $\eta^* = -\eta_r/2$ [14]. As we expected, η^* is negative, so the N-terminal tail is overcharged by the ss RNA. The above calculation is valid if ss RNA wraps around the tail (Fig. 2a) and, therefore, $X_0 > L$. This happens only at $\eta_r/\eta_t < 2$. On the other hand at $\eta_r/\eta_t = 2$, the length of the ss RNA segment in the complex, X_0 reaches the minimum possible value $X_0 = L$ corresponding to stretched ss RNA. At $\eta_r/\eta_t > 2$ both polymers are stretched (Fig. 2b) by the Coulomb self-repulsion, $X_0 = L$, and $\eta^* = \eta_t - \eta_r < -\eta_t$. Thus, at $\eta_r/\eta_t > 2$ the tail is overcharged by ss RNA more than twice.

Until now we assumed that the ss RNA length \mathcal{L} is always larger than X_0 , so that X_0 does not depend on \mathcal{L} . Let us now imagine that we vary \mathcal{L} at fixed L, η_t and η_r . Then for a short ss RNA, $\mathcal{L} < X_0$, (where X_0 is still the optimum value of X found above) the new optimum value of $X = X_{00}$ equals \mathcal{L} (the N-terminal tail consumes all available ss RNA). This means that at $\mathcal{L} < X_0$ the electrostatic energy decreases with growing \mathcal{L} , while for $\mathcal{L} > X_0$ the energy saturates. Thus, complex of ss RNA with an N-terminal tail is most stable if $\mathcal{L} \geq X_0$.

Now we can switch from a single N-terminal tail to the whole brush of 180 tails and a very long ss RNA with the length \mathcal{L} comparable to $180L$. The average distance a between two neighboring tail roots (see Fig. 1b) is typically close to 5 nm. We deal with r_s much smaller than a , so that complexes of the nearest neighbor tails with RNA can be treated separately. This means that long enough ss RNA goes from one tail to another consequently overcharging each of them in the way we calculated above for a single tail (Fig. 1b).

It is easy to show that if $\mathcal{L} < 180X_0$ ss RNA is shared between tails in equal portions $\mathcal{L}/180 < X_0$. In this case the total electrostatic energy still goes down with growing \mathcal{L} . (Here and below we neglect the length of ss RNA per tail necessary to connect the tail roots: it is of the order of $a/2 \ll L$. Indeed, according to Tab. I $L \sim 15$ nm, while $a/2 \sim 2.5$ nm.) On the other hand, when $\mathcal{L} > 180X_0$ and each N-terminal tail gets the length X_0 of ss RNA, the electrostatic energy saturates at low level and does not depend on \mathcal{L} . At this point in order to find optimal length of ss RNA for given tails, we should recall the excluded volume interaction energy, which is smaller than the electrostatic energy, but provides the growth of the free energy with \mathcal{L} at $\mathcal{L} > 180X_0$. Indeed, one should take into account that due to screening the persistence length of the tail-RNA complex is much smaller than the tail length L and the tail-RNA "arches" are not extended as shown in Fig. 1b, but rather tend to make coils. This leads to a noticeable excluded volume interaction. Thus, for given tails the free energy reaches minimum at $\mathcal{L} \simeq 180X_0$. (Similar minimum was ob-

tained earlier for the model of protein charges uniformly smeared on the internal capsid surface [9].) For the theoretical charge inversion ratio \mathfrak{R} we arrive at

$$\mathfrak{R} = \frac{X_0\eta_r}{L\eta_t} = \begin{cases} 1 + \eta_r/(2\eta_t), & \text{when } \eta_r < 2\eta_t \\ \eta_r/\eta_t, & \text{when } \eta_r > 2\eta_t \end{cases}. \quad (2)$$

In Tab. I we calculated the ratio η_r/η_t for the group A viruses using 0.65 nm for the distance between two charges of ss RNA and 0.34 nm for a length of the tail per amino acid. We see that for the most of the viruses $\eta_r/\eta_t \geq 2$ and, therefore, ss RNA is stretched along the N-terminal tails (Fig. 1b), so that a simple way to formulate our results for the length of ss RNA is to say that the total length of ss RNA \mathcal{L} is equal to the total length of the tails $180L$. Substituting values of η_r/η_t from Tab. I in Eq. (2) we arrived at values of \mathfrak{R} listed in Tab. I. We see that most of them are in reasonable agreement with the structural data [16].

This agreement may be interpreted as a result of natural evolution of viruses in the direction of the maximum viral stability. It is desirable, however, to design an *in vitro* experiment, which verifies our predictions. Before suggesting such experiment let us note that although above we discussed only packaging of a single ss RNA molecule in a virus, our conclusions can be extended to the case, where many shorter ss RNA pieces are packaged in the virus. They just continue each other inside the virus and bind proteins together. Our predictions, therefore, can be verified by experiments with a solution of relatively short homo-polymeric ss RNA with the length \mathcal{L} in the range $2L < \mathcal{L} \ll 180L$. We suggest an equilibrium experiment with a series of solutions, which have a varying ratio ρ of the total charges of short ss RNA and capsid capsid proteins. At $\rho \simeq 1$ in equilibrium all ss RNA molecules are used up in viruses, so that there is no free ss RNA. With growing ρ free ss RNA should appear at the critical point $\rho = \rho_c = \mathfrak{R}$, where, according to our theory, free ss RNA molecules and ss RNA molecules inside the virus are in equilibrium. Using short ss RNA permits to vary amount of ss RNA in a virus almost continuously in order to find ρ_c and compare it with \mathfrak{R} .

Let us now discuss the assumptions (i), (ii) and (iii) of our toy model, starting from the assumption (ii), that one can treat the N-terminal tail with a part of it sticking to the internal capsid surface as a free tail. The picture of RNA going along the one side of the tail without wrapping does not seem to be too sensitive to the fact that the other side of the tail sticks to the capsid. This, (together with the fact that in average only 24% of the tail length sticks to the capsid surface) makes (ii) reasonable. The assumption (iii) is more problematic because biological values of $r_s \sim b \sim 1$ nm. They easily satisfy inequalities $r_s \ll L, a$, but do not literally satisfy assumption that $r_s \gg b$. This assumption was important in order to say that ss RNA and N-terminal tail-RNA complex are

stretched and the Coulomb energy dominates the configuration entropy. We argue here that according to numerical simulations [13] for a very flexible polyelectrolyte (with the bare persistence length equal to the Bjerrum length) even for such a small r_s the Coulomb interaction plays a strong role: its persistence length grows three times already at $r_s = 1$ nm. For less flexible polyelectrolyte such as ss RNA or the tail-RNA complex the Coulomb interaction should play even stronger role so that for zero order approximation the configuration of the complex shown in Fig. 2b is reasonable. The assumption (i) that ss RNA behaves as a flexible linear polyelectrolyte is not necessary for a homo-polymeric ss RNA or a generic linear polyelectrolyte used for virus self-assembly in-vitro [4]. On the other hand, for the viral ss RNA, the energy of hydrogen bonds should be optimized together with the electrostatic energy. It seems that effect of such global optimization will not differ much from our result, but this remains to be shown.

Up to now we have dealt with the group A. In the group B charges of the capsid proteins are large but tails are practically neutral so that the theory of Ref. [9] is appropriate. In the group C the charges of proteins and tails are very small but it is possible that for some viruses the internal surface of capsid proteins is positively charged, while the negative charges are on the external surface [18]. In this case, one may also redefine R as ratio of ss RNA charge to the total charge of the internal surface of the capsid and use Ref. [9] to estimate R .

In this paper we focused on T=3 viruses, because they attract most of physicists attention [3, 9, 17]. As we saw many of their capsid proteins have long positive tails. Capsid proteins of some T=1, 4 and 7 viruses also have positively charged tails. Our theory is applicable to them as well. Detailed analysis of these classes is beyond scope of this paper.

In conclusion, the data [7, 8] show that there is a big group of viruses, where practically all positive charges of a capsid protein are concentrated in a long and flexible N-terminal tail. For a given length and charge of the tail we optimized the length of the ss RNA genome by searching for minimum of free energy of the virus. We arrived at the very simple result that a virus is most stable when the total length of ss RNA is close to the total length of the tails. This result is in reasonable agreement with the viral structural data [7, 8]. This may be interpreted as a result of evolution in the direction of viral stability.

We are grateful to M. Rubinstein for valuable contribution during initial stage of this work and to Z. Wang and M. Rubinstein for showing us their unpublished computer simulations of similar to Fig. 2a confirmations of diblock polymer molecule consisting of two oppositely charged

blocks. We appreciate useful discussions with R. Bruinsma, A. Yu. Grosberg, T. T. Nguyen, and I. Rouzina. B. I. S. acknowledges hospitality of Santa Barbara KITP and Aspen Center of Physics, where this work was started.

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- [1] Encyclopedia of Virology Plus CD-ROM. Edited by R. G. Webster and A. Granoff. Academic Press (1995).
 - [2] A. Scheneemann, Annu. Rev. Microbiol., **60**, 51 (2006).
 - [3] R. F. Bruinsma, Eur. Phys. J. E, Soft Matter, **19**, 303 (2006).
 - [4] J. B. Brancroft, E. Heibert and C. E. Bracker, Virology **39**, 924 (1969).
 - [5] P. P. Hung, C. M. Ling and L. R. Overby, Science. **166**, 1638 (1969).
 - [6] K. W. Adolph and P. J. Butler, Philos Trans R. Soc Lond B (Biol Sci.) **276**, 113 (1976).
 - [7] <http://www.ncbi.nlm.nih.gov>.
 - [8] <http://viperdb.scripps.edu>.
 - [9] P. van der Schoot and R. Bruinsma, Phys. Rev. E **71**, 061928 (2005).
 - [10] S. J. Flint, L. W. Enquist, V. R. Racaniello and A. M. Skalka, *Principles of Virology* 2nd ed (ASM, Washington DC, 2004).
 - [11] T. Odijk, J. Polym. Sci., Polym. Phys. Ed. **15**, 477 (1977).
 - [12] J. Skolnick and M. Fixman, Macromolecules **10**, 944 (1977).
 - [13] T. T. Nguyen and B. I. Shklovskii, Phys. Rev. E **66**, 021801 (2002).
 - [14] For similar but not identical problem of flexible polyelectrolyte wrapping oppositely charged rigid cylinder, this result was previously obtained in Ref. [15].
 - [15] T. T. Nguyen, B. I. Shklovskii, Phys. Rev. Lett. **89**, 018101 (2002); H. Borodjerdi, Y-W. Kim, A. Naji, R. R. Netz, X. Shlagberger, A. Serr, Phys. Rep. **416**, 129 (2005).
 - [16] One can see strong deviations from our predictions for viruses in the third, fourth and the last three lines of the group A. They have anomalously long tails with strongly non-uniform distribution of charges in the tail [7, 8]. Heavily charged domains occupy roughly speaking only half of the tail length. Because of the salt screening neutral pieces are not stretched by the Coulomb repulsion of the neighboring charged domains and acquire coil-like configurations. As a result, a charged tail has twice larger linear charge density. Therefore, according to Eq. (2) predicted \mathfrak{R} should be roughly speaking twice smaller, in a reasonable agreement with R . In essence in these cases, we can not neglect the configurational entropy.
 - [17] J. Rudnick and R. Bruinsma, Phys. Rev. Lett. **94**, 038101 (2005).
 - [18] T. T. Nguyen and R. F. Bruinsma attracted our attention to this possibility.